REMARKS / ARGUMENTS

The office action dated August 2, 2011 has been carefully considered. It is believed that the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Claim objections

The Examiner's objections on page 3 were reviewed and the required corrections of the status of the claims were made._Claims 5, 8-21, 25, 32, 37, 39-40 and 45-46 are corrected according to Examiner's remarks on page 3 and are identified as "previously presented". Claims 29-31 and 47 are corrected according to Examiner's remarks on page 4 and are identified as "Withdrawn".

35 USC 112

On page 4 the Examiner rejects claims 23-26, 34, 45 and 46 as being indefinite for failing to distinctly claim and point out the subject matter of the invention.

The Applicant believes that the description of the experiments makes it clear that the increase of the ionic strength is achieved by increasing the salt concentration in the elution buffer comparing to the washing buffer. See Examples [0263-0351] where the washing buffer contains 100mM KCl and the washing buffer contains 0.3M or 0.4M KCl. However, the Applicant agrees that the claims will be clearer if the Examiner's suggestion is included and amends claim 23 as follows:

23. (currently amended) The method according to claim 22, wherein the ionic strength of the system is increased ~~with~~ by increasing the concentration of a chemical agent.

The Applicant believes that with this amendment of claim 23 claims 25, 26 and 34 have proper antecedent basis.

Regarding claims 45 and 46 and Examiner's remarks on page 4 (last paragraph), the Applicant respectfully points to paragraphs [0256-0261] of the instant application as describing how a "method for separating first ligand from second ligand in an affinity matrix system" can provide for ligand-ligand association as a putative cause for a disease to be identified.

On page 5 the Examiner rejects claims 25, 26 and 34 because the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention. Specifically, the Examiner considers the limitations of claims 25, 26 and 34 to be contradictory to Applicant's own terminology which defines a concentration of at least 100mM, instant application [0144-0145]. Applicant respectfully submits that claims 25, 26 and 34 state "change of concentration" not "concentration". It is clear from the description of the invention that the elution of the first ligand is performed with a buffer with higher salt concentration than the washing buffer. Thus the "change of concentration" denotes the difference of salt concentration of KCl in washing buffer and lysis buffer. See Examples [0263-0351] where the washing buffer contains 100mM KCl and the washing buffer contains 0.3M or 0.4M KCl. As an example, see the description of the experiment in [0263-0279] , where Lysis Buffer and the washing buffer contain 100 mM

KCl but the Elution buffer contains 0.4M KCl. In this case the "change of concentration" is 0.3M.

35 USC 103(a)

The Examiner rejects claims 1-26, 32, 34, 35, 37-40, 45 and 46 as being unpatentable over Seraphin et al., US 2002/0061513 as evidenced by Fink et al., Biochemistry, 1994, 33 (41), in view of Kellogg et al., Methods in Enzymology, 2002, vol.351, pp172-183 and Hentze et al., WO 2000/053779, published 09/14/2000.

According to the Examiner's response on page 9, Seraphin et al. paragraph [0029], teaches the use of conventional elution techniques such as varying pH or salt concentration for recovering the protein from the support material. After carefully reviewing paragraph [0029], the Applicant respectfully objects that these elution techniques are explicitly described for separating the second ligand from the support material. This way, Seraphin et al, paragraph [0029] teach away from the present invention.

On page 10 the Examiner refers to Kellogg et al., page 183 last paragraph where Kellogg et al. teaches that protein interactions that can be disrupted with salt concentration above 0.5M are most likely to interact *in vivo*. However, Kellogg et al. et al. does not distinguish between permanent and transient protein bonds as it is described in the instant patent application, See "Background Of The Invention" [0002] and [0004-0013]. Most importantly, the teaching that

protein interactions that can be disrupted with salt concentration above 0.5M are most likely to interact *in vivo* leads away from the present invention. Indeed, the inventors have found that the transient protein bonds can be disrupted by elution with 0.3M or 0.4M salt concentration, without disrupting the permanent protein bonds. Devising a method for disrupting the transient protein bonds while the permanent protein bonds remain intact is the main feature of the instant patent application. In addition, since the increase of salt concentration in the elution buffer above 0.5M leads to partial or complete separation of the second ligand from the affinity column, see [0329], the Applicant respectfully points that Kellogg et al. et al., page 183 last paragraph, teaches away from the present invention.

On page 10 The Examiner opines that the conventional elution techniques, e.g. varying pH or salt concentration, taught by Seraphin et al., and as evidenced by Kellogg et al. and Fink et al. would inevitably lead to disrupting the in vivo associated protein complexes.

However, since Seraphin et al. paragraph [0029] teach that conventional elution techniques such as varying pH or salt concentration lead to separating the second ligand from the support material, person of ordinary skill in the art would find it

contradictory to Kellogg et al, page 179 second paragraph, i.e. that varying pH or salt concentration may lead to removal of the associated proteins "leaving the original protein still bound" to the support material. Thus, person of ordinary skill in the art would not find it useful or desirable to combine the two contradictory teachings of Seraphin et al and Kellogg et al.

On page 10, third paragraph, of the response the Examiner opines that Kellogg et al. teach the associated proteins to be eluted with high salt buffer, leaving the original protein still bound to the antibody on the column. The Applicant has carefully reviewed the entire publication and respectfully disagrees and points out that actually all the methods described in Kellogg et al., Methods in Enzymology, 2002, vol.351 aim at removal of the entire multiprotein complexes from the affinity column. As an example see the experimental protocols on pages 175, 177-178. The teaching of removal of removal of the entire multiprotein complex from the column teaches away from the present invention.

Furthermore, Kellogg et al, page 179 second paragraph states that the removal of the associated proteins "leaving the original protein still bound" to the support material happens "in some cases". It is clear that Kellogg et al. consider the separation of the associated proteins as a failure to isolate the entire protein complex. Indeed, the experimental protocol on pages 177-178, specifically describes the 0.3-1M KCl elution as targeting the separation of the second ligand, i.e. the protein that binds the antibody, from the support material. Moreover, in the same experimental protocol on page 178, fourth paragraph, Kellogg et al. state that some interacting proteins remain bound to the column even after elution with 1M KCl, in which case elution with 2.5M urea is recommended. This clearly indicates that Kellogg et al. teach the separation of the entire protein complex, including the second ligand, from the affinity column which teaches away from the invention in the instant patent application.

On the other hand, one of ordinary skill in the art following the teachings of Seraphin et al. would try to isolate the first ligand and second ligand together and present the result as a strong

proof for their interaction/association. Since both Seraphin et al. and Kellogg et al. have as a clearly defined goal to isolate all the subunits of the multiprotein complex, a person of ordinary skill in the art would reasonably expect that elution with high salt buffer would lead to disintegration of the complex and the removal of the second ligand from the affinity column. Again, on page 179, second paragraph, Kellogg mentions the separation of the associated proteins as a side effect but not as a novel method devised for solving a specific problem.

Most importantly, Kellogg et al. do not show any result that proves that the described conditions, i.e. 0.3-1M KCl elution of the immobilized protein complex leads to separating the first ligand from the immobilized second ligand. It is possible that the second ligand might have been eluted but not detected by Kellogg et al. which is in no way a rare occurrence in experiments involving isolation and detection of proteins. Another possibility is that the "associated proteins" were bound nonspecifically to the support material, e.g. Agarose or Sepharose, but not to the protein that binds the antibody. The Applicant respectfully requests for a proof that the proteins that Kellogg et al. consider "associated proteins" were associated with the original protein that binds the antibody and that this protein remained immobilized after the high salt elution.

In contrast, the instant application includes an experiment that clearly proves that the first ligands were separated from the second ligand that remained immobilized on the affinity column. See Figure 11 and paragraphs [0351-0352]. Briefly, after the high salt elution with 0.3 M KCl and separation of the first ligands, another elution with 5 mM EGTA and 0.5% SDS was performed in order to demonstrate the presence of the second ligand which remained immobilized on the column after the high salt elution.

On page 11 the Examiner states that a person of ordinary skill in the art would combine the teachings of Kellogg et al and Seraphin et al. because it would be desirable to separate first ligand from the immobilized second ligand. The Applicant respectfully objects that neither Kellogg et al. nor Seraphin et al. teach the usefulness and desirability of separating the first ligand from the immobilized second ligand. The Applicants were first to propose and demonstrate the usefulness and desirability of separating the first ligand from the immobilized second ligand, i.e. the elimination of the dynamic range problem [0009-0015 and 0077- FIG. 21] in the present application. The Applicant respectfully submits that the priority documents mentioned by the Examiner, do not suggest the usefulness and desirability of separating the first ligand, which has been associated in vivo with the second ligand before the start of the experiment, from the second ligand which remains bound to the affinity column. The Applicants were the first to propose and demonstrate that an in vivo formed protein complex can be immobilized on an affinity matrix in such a way that would allow for the selective separation of the proteins (i.e. first ligands) that interact with the immobilized affinity tagged protein (i.e. second ligand) while the latter remains immobilized. Prior to the present application, a person skilled in the art would not expect to be able to separate transiently interacting substoichiometric proteins from permanent protein members of such an *in vivo* formed complex. One skilled in the art would try to perform the present invention only after following the teaching in [0219] of this application that states "Permanent protein complexes are held together mainly by hydrophobic forces and transient ligand complexes are held together in large part by electrostatic attractions".

Prior to the present application, a person skilled in the art would not be motivated to disrupt electrostatic interactions from the immobilized multi-protein complex described in Seraphin et al

based on the disclosure of Kellogg et all. Seraphin et al not only do not suggest the desirability of separating the first ligand from the immobilized second ligand but teach an entirely opposite method, i.e. isolating an intact multiprotein complex. The inventors of the claimed invention were the first to suggest and demonstrate that elimination of the dynamic range problem by separating the substoichiometrically interacting proteins (i.e. first ligand) from the immobilized fusion protein (i.e. second ligand that remains immobilized) is possible and desirable (see patent application [0004-0015] and [0057-0067]). The cause for the failure to detect substoichiometrically interacting proteins was not obvious at the priority date of the invention. For example, the substoichiometric interactors (i.e. first ligands) could have just been absent after immobilizing the protein of interest (i.e. second ligand) on affinity matrix or, they could have been present at amounts that do not allow their proper analysis and identification.

The applicants were the first to identify the cause for this failure, i.e. high dynamic range, and have designed a method which solves the problem. Three non-obvious concepts are incorporated in the invention:

- transient interactors (i.e. first ligands) are present after the immobilization of the protein of interest (i.e. second ligand) on the affinity matrix but they are not detected because of the low stoichiometry (high dynamic range);

- transient interactors have to be separated from the high abundance affinity tagged protein and analyzed separately; in addition, the separation should not lead to separation of any other associated high abundance protein from the affinity tagged protein.

- the separation of in vivo formed transient protein complexes, after their immobilization on affinity column, can be achieved by increasing the ionic strength;

On pages 10 and 11 of the response the Examiner opines that a person having ordinary skill in the art would have found the conditions for performing the present invention, i.e. the change of concentration, by performing a gradient salt elution taught by Kellogg et al., in a method taught by Seraphin et al. Further, the Examiner opines that as evidenced by Fink et al. a person having ordinary skill in the art would have known that salt elution disrupts the protein bonds associated by electrostatic forces. The Applicant respectfully objects that by performing a gradient elution as taught by Kellogg et al., the first ligand will be unnecessarily distributed in many fractions and the probability of its detection will be much lower than collecting a single fraction by performing a single elution. Thus, a person having ordinary skill in the art would not have arrived at the claimed conditions, i.e. salt concentrations, by routine optimization. The Applicant respectfully reiterates that prior to the teachings included in the present invention, a person having ordinary skill in the art would have had no motivation to separate the first ligand from the immobilized second ligand. Indeed, if a person having ordinary skill in the art would have wanted to demonstrate that first ligand and second ligand form a protein complex, he would have performed the method of Seraphin et al. which is very straightforward. On the other hand, if a person having ordinary skill in the art would have wanted to isolate the first ligand as a single protein in order to study its properties he would have used classical affinity purification or any method for purification via an affinity tag such as purification of GST fusion protein as described in Kellogg et al., Page 175.

Furthermore, the Applicant respectfully submits that two ligands can be associated in vivo by electrostatic bonds but it does not necessarily mean that they can be separated from one another by increasing the ionic strength of the medium. As evidenced in "Structural basis of

transcription: RNA polymerase II at 2.8 angstrom resolution" by Cramer P, Bushnell D, Kornberg R. (Science, 2001, http://www.sciencemag.org /cgi/reprint/292/5523/1863.pdf, previously submitted), Table 2 - "Subunit interactions", Rpb1 forms 6 salt bonds, i.e. electrostatic bonds, with Rpb2, 5 salt bonds with Rpb5, and three salt bonds with Rpb6 and Rpb8. In total, there are 45 electrostatic bonds between the 12 subunits, i.e. Rpb1, Rpb2...Rpb11, Rpb12, of the permanent protein complex RNA polymerase II. After immobilizing the RNAP II complexes on affinity matrix by affinity tagged Rpb1, the other 11 subunits of the core RNA Polymerase II complex are present at approximately the same molar amount as Rpb1. This amount is much higher (with at least an order of magnitude) than the amount of the transcription factors. Yet, the 11 subunits of the core RNA Polymerase II complex (other than the immobilized Rpb1), are not eluted upon increasing the ionic strength (see the results in Figures 3, 4, 6, 7, 8, 10, 11, 12). If one of ordinary skill in the art follows the teachings of Kellog et al., Seraphin et al. and Fink et al., he would expect Rpb2, Rpb5, Rpb6 and Rpb8 to be eluted/ desorbed upon increasing the ionic strength.

Although it is probably not necessary to provide explanation, Applicant believes there are at least two reasons: (a) the electrostatic bonds might be inaccessible to the small ions (resulting from the increased ionic strength) and/or (b) beside the electrostatic bonds, the two ligands can be associated by other bonds, i.e. hydrophobic bonds, and even if the electrostatic bonds are disrupted, the two ligands can remain bound to each other. Yet, in both cases the association between the two ligands can be most appropriately described as "associated by electrostatic bonds" because the energy of the electrostatic bonds, especially the Coulomb forces, is much bigger than the energy of the non-electrostatic bonds.

The fact that Rpb2, Rpb5, Rpb6 and Rpb8 remain bound to the immobilized Rpb1, despite the separation of 50 other proteins, argues strongly that the present invention is not obvious. (In other words, if step 1(d) is obvious, i.e. that two ligands that associate by electrostatic bonds can be separated be destroying the electrostatic bonds, why are not the other 11 subunits of the core RNA Polymerase II complex eluted and detected upon increasing the ionic strength?)

In addition, the Applicant respectfully points that elutions are performed at higher salt elutions which disrupt not only the electrostatic bonds between the first and second ligand but the electrostatic bonds between the affinity column and the affinity tagged second ligand. See [0301] of this application where 0.7 M potassium acetate is used to separate the first ligand from the immobilized second ligand. See the above example of the ubiquity of electrostatic bonds between proteins. Applicants respectfully submit that for the presently claimed methods, not only is the bond between the first ligand and the second ligand a protein-protein bond, but the bond between the second ligand and the affinity column is also a protein-protein bond. Thus, a person having ordinary skill in the art would reasonably expect that disruption of one protein-protein bond, i.e. between the first and second ligand, would lead to the disruption of another protein-protein bond, i.e. between the second ligand and the affinity column.

As a further evidence for the non-obviousness of the invention, the Applicant respectfully points out that the present invention solves an outstanding and long-recognized problem in the field of detecting protein-protein interactions, i.e. high dynamic range problem. This is demonstrated by the huge difference between the results obtained by using the method of the present invention and the prior art. As an example, Gavin et al. Nature (2002), previously submitted, used the

method of Rigaut on a large scale (1,739 protein purifications) and, in particular, performed 3 protein purifications by using an affinity tag fused to 3 different subunits (Rpb3, Rpb7 and Rpb9) of RNA polymerase II which is a permanent complex of twelve subunits (Rpb1-Rpb12). Since the purpose of the method of "Rigaut" is to isolate the entire in vivo formed protein complex, i.e. second ligand and first ligand(s), they detect only 7 proteins that interact with the permanent complex (See Supplementary table S1). By contrast, by using the method of our invention, we were able to identify more than 70 additional interacting proteins (paragraph [0357] of this patent application and Figures 3-12). Moreover, Gavin et al. Nature (2002) demonstrates that a team of 38 skilled scientists performed 1,739 protein purification but failed to realize that separating of the first ligand from the immobilized second ligand would greatly improve the final result. This argues for the non-obviousness of the invention.

The same failure is demonstrated in Krogan et al. Nature (2006), http://www.ncbi.nlm.nih.gov/pubmed/16554755, where 52 scientists processed 4,562 tagged proteins, i.e. second ligands, in order to detect the interacting proteins, i.e. first ligands, and again did not realize the desirability and usefulness of separating the first ligand from the immobilized second ligand. The Applicants were the first to propose and demonstrate that an in vivo formed protein complex can be immobilized on an affinity matrix in such a way that would allow for the selective separation of the proteins (i.e. first ligands) that had been associated with the immobilized affinity tagged protein (i.e. second ligand) while the latter remains immobilized.

Regarding claim 34, the Applicant respectfully submits that KCl is not a biomolecule and that it affects non-selectively many protein-protein pairs as shown in Figures 3-12, i.e. around 70 different proteins were separated from the immobilized second ligand by using one agent. In

contrast, claim 34 clearly states that the "biomolecule is identified as a drug or pre-drug by its capability to separate selectively the first ligand from the second ligand and/or affect selectively the separation of the first ligand from the second ligand" (Emphasis added). Paragraphs [0249 - 0261] of Description of the invention describe the difference between the non-selective effect of increasing the ionic strength on any electrostatically bound protein-protein pair and the selective effect of biomolecule on a specific protein-protein pair. Figure 19 illustrating the difference between selective and non-selective separation of protein pair. In addition, claim 34 defines the biomolecule as capable to separate two proteins at "the change of the concentration of the chemical or biomolecule is below 30 mM". See Examples [0368- 0380] which includes a detailed description of an experiment designed to determine the effect of a drug on the complex. The Applicant believes that this disclosure is enough to enable a person having ordinary skill in the art to perform the method.

On page 12 of the response the Examiner rejected claims 45 and 46 under 35 USC 103(a) as being obvious having regard to Seraphin et al. in view of Kellogg et al. as applied to claims 1-26 above, and further in view of Patent 6,610,508 to Hentze et al. As discussed above, Seraphin et al. in view of Kellogg et al. do not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. The Examiner alleges that a person skilled in the art would have found it obvious to modify the methods of Seraphin et al. and Kellogg et al. to include the step of identifying protein-protein interactions for detecting Alzheimer's disease. The fact that Hentze discloses a step of identifying protein-protein interactions in order to detect disease states including Alzheimer's

Disease does not correct for the deficiencies discussed above of Seraphin et al. in view of Kellogg et al.

Applicant respectfully request that the rejections under 35 USC 103(a) be withdrawn and submits that claims 1-26, 29-32, 34-52 are inventive.

In view of the foregoing comments and amendments, we respectfully submit that the application is in order for allowance. Should the Examiner deem it beneficial to discuss the application in greater detail, he is kindly requested to contact the undersigned by telephone at (519) 837-9427 at his convenience.

Respectfully submitted,

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